Relationship of Venom Effects to Venom Antigen and Antivenom Serum Concentrations in a Patient With *Crotalus atrox* Envenomation Treated With a Fab Antivenom

Steven A Seifert, MD*
Leslie V Boyer, MD‡
Richard C Dart, MD, PhD§
R Stephen Porter, PharmD||
Lena Sjostrom, PhD||

From the Department of Emergency Medicine, Kino Community Hospital, and the Arizona Poison and Drug Information Center,† Tucson, AZ; the Rocky Mountain Poison and Drug Center, Denver, CO;§ and Therapeutic Antibodies, Nashville, TN, and London, United Kingdom.||*

Received for publication May 20, 1996. Revisions received September 12, 1996, and January 14, 1997. Accepted for publication March 17, 1997.

Presented in abstract form at the North American Congress of Clinical Toxicology Annual Meeting, October 1996.

Funded in part by Therapeutic Antibodies, Incorporated.

Study objective: To described the association among venom antigenemia, serum antivenom concentrations, and venom effects in a 53-year-old woman who was bitten by a Western diamondback rattlesnake *(Crotalus atrox).*

Methods: The patient was enrolled in a multicenter trial of an investigational Fab antivenom. Her clinical condition and coagulation parameters were monitored for 2 weeks after the bite.

Results: After antivenom administration, the progression of the venom's effects was arrested. The antivenom reversed some local venom effects, caused venom antigens to disappear from the blood, and resolved the patient's profound thrombocytopenia (before antivenom, 12,000/mm^3; 1 hour after antivenom, 227,000/mm^3). Local venom effects recurred twice in the 24 hours after antivenom administration but were easily managed with additional Fab antivenom. Venom antigenemia was detected on days 5 and 8 after the initial treatment and was accompanied in one instance by the new onset of hypofibrinogenemia (119 mg/L) that resolved spontaneously and in both instances by renewed profound thrombocytopenia. Repeat Fab antivenom doses on days 6 and 9 were followed by increases in platelet count (from 16,000 to 40,000/mm^3 and from 11,000 to 20,000/mm^3, respectively) and by the reduction or disappearance of venom antigenemia. The patient sustained no significant bleeding complications, and all laboratory values had returned to normal 2 weeks after the bite.

Conclusion: Initial control of local symptoms and coagulopathy was prompt after the administration of Fab antivenom. Repeat doses during the 24 to 36 hours after a bite may be necessary for local control. Recrudescence of coagulopathy was likely due in part to renewed venom antigenemia after clearance of Fab antivenom. The role of Fab antivenom in the treatment of recurrent coagulopathy requires further study.

[Seifert SA, Boyer LV, Dart RC, Porter RS, Sjostrom L: Relationship of venom effects to venom antigen and antivenom serum concen-

trations in a patient with *Crotalus atrox* envenomation treated with a Fab antivenom. *Ann Emerg Med* July 1997;30:49-53.]

INTRODUCTION

Crotalid venom may produce a coagulopathy consisting of thrombocytopenia, hypofibrinogenemia or both. In many cases, patients exhibit prolonged coagulopathic manifestations, with coagulopathies persisting as long as 2 weeks after envenomation.[1-6]

Recurrence of coagulopathy after complete normalization has also been reported.[7] These effects have not previously been related to circulating serum concentrations of antivenom or venom antigens in crotalid envenomation. We report a case of recurrent local and coagulopathic venom effects linked to reciprocal serum concentrations of venom antigens and of an investigational Fab antivenom (CroTAb polyvalent crotalid antivenin, ovine Fab; Therapeutic Antibodies).[8]

CASE REPORT

A 53-year-old woman presented to the ED 2 hours after being bitten in the first web space of the left hand by a snake identified by trained fire department personnel as a large Western diamondback rattlesnake *(Crotalus atrox)*. Edema had progressed to a point just below the shoulder. Initial coagulation parameters were notable for thrombocytopenia (Table).

The patient gave informed consent for enrollment in a multicenter clinical trial of Fab antivenom, which had received institutional review board approval. She was given six vials (750 mg/vial) of Fab antivenom infused intravenously over 1 hour in keeping with the investigational protocol. Proximal progression of edema and circumferential enlargement of the patient's hand, forearm, and arm ceased during the infusion. The patient's platelet count returned to normal within an hour of the end of Fab antivenom administration. Fibrin degradation products (FDPs) appeared 12 hours after the initial treatment.

Local symptoms recurred 6 and 14 hours after the initial Fab antivenom dose. Two additional vials of Fab antivenom

Table.
Timing, antivenom doses, coagulation parameters, and Fab and serum venom concentrations.

Time							
Days	Hours	Antivenom Dose (Vials)	Platelet Count (per mm^3)	Fibrinogen Concentration (µg/L)	FDPs	Free Fab (mg/L)	Venom Concentration (µg/L)
	−1		12	227	Undetectable	ND	ND
	0	6					
	1		227	325	Undetectable	740*	17
	6		289	326	Undetectable	130	0
	8	2					
	12		246	347	Detectable	140	0
	14	2					
	18		277	320	Detectable	260	0
	36		221	363	Detectable	52	0
5	135		11	119	Detectable	10	7
6	145		16	212	Detectable	7	10
	154	2					
	155		40	—	—	—	—
7	160		58	—	—	—	—
	190		25	—	—	—	—
8	195		23	214	Detectable	12	2
9	205		11	—	—	7	0
	214	2					
	215		20	—	—	325	0
	230		34	—	—	37	0
10	255		47	—	—	16	0
13	325		220	275	Undetectable	2	0

—, not determined.
*Total Fab; insufficient sample left for analysis of free Fab.

were given after each recurrence. Proximal progression ceased during each 1-hour infusion period, and upper-arm circumference decreased by 3 cm on the first occasion and 4.5 cm on the second, over a 6- to 8-hour period following infusion. No further local recurrences were noted. The platelet count remained above 220,000/mm^3, no other coagulation parameter abnormalities were noted during the patient's 36-hour hospitalization.

The patient returned for clinic follow-up 5 days (135 hours) after the bite. Laboratory evaluation was notable for recurrence of profound thrombocytopenia, mild hypofibrinogenemia, and persistence of FDPs.

The administration of two vials of Fab antivenom on day 6 produced a prompt increase in platelets, but this increase was less pronounced than the response after the first dose of antivenom. Two days later, the platelet count had again decreased. The platelet count again responded to two more vials of Fab antivenom, but this response was still smaller than the second.

The patient's fibrinogen concentration was normal throughout hospitalization but was noted to increase sharply after the initial dose of Fab antivenom. FDPs became detectable shortly after admission and remained so until discharge. The fibrinogen concentration was mildly decreased at the first follow-up visit and associated with continued presence of FDPs. The fibrinogen concentration spontaneously returned to normal before antivenom was given on day 6. FDPs remained detectable for 3 more days, but the fibrinogen concentration remained normal. The patient experienced no spontaneous hemorrhage, and all laboratory values had returned to normal by the end of 2 weeks.

Free Fab antivenom and venom antigen concentrations were later assayed in the specimens drawn during the course of the patient's care. The three blood specimens obtained between 150 and 200 hours after antivenom infusion were inadvertently discarded before Fab antivenom and venom antigen analysis. Venom antigens were detectable in the earliest blood specimen but were undetectable in the specimen obtained 1 hour after the initial dose of Fab antivenom. They remained undetectable during the local recurrences and through discharge. Venom antigens were detectable on the first follow-up visit, when Fab antivenom concentrations had decreased and on days 6 and 8, also in association with low Fab antivenom concentrations, after which time venom antigens were no longer detectable.

DISCUSSION

In our patient, the progression of local findings ceased promptly after Fab antivenom administration and recurred twice during the first 24 hours. In both instances, administration of additional Fab antivenom was associated with prompt cessation of progression and some reversal of local findings. In preliminary work, a relatively short half-life of 2.5 hours was found for free Fab.[8] Because no serum venom antigens were detected, we postulate that a depot of unneutralized venom formed at the bite site and caused the recurrence of local symptoms once circulating free Fab antivenom decreased below a protective concentration. Progression of local symptoms can persist in the 24 to 36 hours after an untreated bite, as well as bites treated with whole IgG antivenom.[9,10]

Fibrinogenolysis, destruction of other coagulation factors, and thrombocytopenia are common features of crotalid envenomation.[9-11] The prompt and dramatic initial increase in platelet count, as well as the increase in serum fibrinogen concentration after the initial Fab antivenom dose in our patient, suggests neutralization of venom, with reversal of its coagulopathic effects, by the Fab antivenom. The speed of the platelet response suggests this was most likely the result of reversal of venom-induced aggregation. After this initial response, we noted what appeared to be delayed hypofibrinogenemia and recurrent thrombocytopenia.

In our patient's case, fibrinogenolysis was most likely an ongoing process beginning shortly after envenomation, as evidenced by the initial increase of serum fibrinogen concentration immediately after administration of Fab antivenom and the early appearance of FDPs. Because FDPs did not clear before the patient's discharge, it is uncertain whether complete cessation of fibrinogenolysis occurred. The new onset of hypofibrinogenemia at 5-day follow-up therefore reflects not a delayed coagulopathy but a prolonged or recurrent coagulopathy and was linked temporally to the reappearance of detectable serum venom antigens. Spontaneous resolution occurred in this case.

We noted recurrent thrombocytopenia after apparent resolution following the initial dose of Fab antivenom. As with fibrinogen, the process may have been occurring at a rate that permitted a return to normal levels through the period of hospitalization once venom-induced aggregation had been reversed. However, recurrence of thrombocytopenia was also linked temporally with the recurrence of serum venom antigens. In our patient, the association of recurrent thrombocytopenia and hypofibrinogenemia with recurrent venom antigenemia, as well as the response of platelet count to Fab antivenom in each instance, suggests recurrent venom effects. The similarity of early and late coagulopathies also supports this likelihood. A nonspecific cause of coagulation parameter consumption would be expected to result in a generalized decrease in coagulation compo-

nents, not just the particular factors involved early in the course, and not to the same extent. However, because no specimens were taken between discharge and 5 days, it is uncertain exactly when the first recurrence occurred and whether it was entirely the result of ongoing venom activity or whether it was in part the result of other modes of increased platelet destruction. The progressively lesser magnitude of the response in platelet count to late Fab antivenom indicates decreased direct effectiveness of Fab antivenom, a context of progressively depleted platelet availability, or a combination of such processes.The development of human antisheep antibodies could impair late Fab antivenom neutralization of venom. However, the initial recurrence in our case occurred on day 5, a significantly shorter period than the development of human antimouse antibodies, which are known to be produced within 7 to 10 days of exposure.[12] Antibodies to ovine Fab antivenom require more than 3 weeks in the rabbit and 6 weeks in the baboon to develop.[13]

Possible mechanisms of decreased platelet availability included decreased responsiveness of platelets to deaggregation, increased rates of platelet destruction, and depleted or impaired marrow stores. Deaggregated platelets in contexts other than snakebite have been shown to have shortened half-lives.[14] Increased destruction could also be due to sequestration of platelets in damaged tissue or sensitization resulting from vascular endothelial damage. Simon and Grace[15] demonstrated sequestration of platelets in the damaged tissue at the sites of pit viper bites in rabbits. Increased consumption of platelets resulting from damaged vascular endothelium is reported to occur in *C horridus horridus* envenomation.[2] Decreased regeneration from marrow injury has not been demonstrated after envenomation by *C horridus horridus*[5] or *Crotalus ruber ruber*[3] (the red diamond rattlesnake).[3]

Recurrence of previously corrected coagulopathy has followed complete resolution of coagulopathy in otherwise adequately treated patients.[7,16,17] Hardy reported two cases of recurrent thrombocytopenia in patients bitten by the northern blacktail rattlesnake (*Crotalus molossus molossus*). In the first case, the patient was treated with 30 vials of antivenom (polyvalent antivenom; Wyeth) with complete normalization before recurrence. In the second case the patient received 21 vials of antivenom and had near-normalization of platelet count before recurrence.[7] Evidence linking the decrease of antivenom concentrations with recurrent venom antigenemia and recurrent coagulopathy has been demonstrated in noncrotalid envenomation. Gillissen[16] demonstrated recurrent thrombocytopenia and hypofibrinogenemia in an *Echis 'pyramidum'*–complex envenomation linked temporally to the decrease of antivenom concentrations in the blood. In this case, venom antigens were still detectable 13 days after envenomation. Ho and colleagues[17] showed an inverse relationship between venom and antivenom (horse IgG) titers in the bite of the Malayan pit viper (*Calloselasma rhodostoma*).[17] Thus it appears that a depot of venom components may remain in the body for weeks after envenomation and is capable of producing prolonged or recurrent coagulopathic effects when protective serum concentrations of antivenom are lost.

It is unknown whether the Fab antivenom–venom complexes can dissociate and serve as a source of recurrent venom antigenemia. Recurrence of increased digoxin concentrations and toxicity has been demonstrated in patients with digoxin toxicity who were treated with an ovine digoxin-specific Fab (Digibind; Glaxo Wellcome).[18] This phenomenon appears to be due to redistribution of digoxin from the extracellular space after the decrease of free Fab concentrations. Dissociation of circulating Digibind–digoxin complexes, however, does not appear to occur, even in patients with impaired kidney function and prolonged circulation of Digibind– digoxin complexes.[19]

In our patient, neutralization of the local effects of North American crotalid envenomation was rapidly achieved with an investigational Fab antivenom but recurred during the 24 hours following the bite, and local recurrence was not associated with recurrent venom antigenemia. In patients treated with Fab antivenom, periodic dosing may be necessary in the 24 to 36 hours following the bite for control of persistent local symptoms. Coagulopathy was likewise rapidly controlled, but recurrent venom antigenemia appears to have been responsible, at least in part, for recurrent thrombocytopenia and hypofibrinogenemia. The clinical implications of a recurrent mild or isolated coagulopathy and the usefulness of late Fab antivenom administration is unclear and merits additional study.

REFERENCES

1. Hasiba U, Rosenbach LM, Rockwell D, et al: DIC-like syndrome after envenomation by the snake *Crotalus horridus horridus. N Engl J Med* 1975;292:505-507.

2. Furlow TG, Brennan LV: Purpura following timber rattlesnake *(Crotalus horridus horridus)* envenomation. *Cutis* 1985;35:234-236.

3. Lyons WJ: Profound thrombocytopenia associated with *Crotalus ruber ruber* envenomation: A clinical case. *Toxicon* 1971:237-240.

4. Budzynski AZ, Pandya BV, Rubin RN, et al: Fibrinogenolytic afibrinogenemia after envenomation by Western diamondback rattlesnake *(Crotalus atrox). Blood* 1984;63:1-14.

5. Tallon RW, Kock KL, Barnes SG, et al: Envenomation coagulopathy from snake bites [letter]. *N Engl J Med* 1981;305:1347.

6. La Grange E, Russell FE: Blood platelet studies in man and rabbits following Crotalus envenomation. *Proc West Pharm Soc* 1970;13:99-105.

7. Hardy DL, Jeter M, Corrigan JJ: Envenomation by the Northern blacktail rattlesnake *(Crotalus molussus molussus)*: Report of two cases and the in vitro effects of the venom on fibrinolysis and platelet aggregation. *Toxicon* 1982;20:487-493.

8. Dart RC, Seifert SA, Carroll L, et al: Human trial of an affinity purified antibody fragment for snake venom poisoning [abstract]. *Vet Hum Toxicol* 1994;36:363.

9. Russel FE: *Snake Venom Poisoning,* ed 2. Great Neck, NY: Scholium, 1983.

10. Burch JM, Agarwal R, Mattox KL, et al: The treatment of crotalid envenomation without antivenin. *J Trauma* 1988;28:35-43.

11. Ouyang C, Teng C-M, Huang T-F: Characterization of snake venom components acting on blood coagulation and platelet function. *Toxicon* 1992;30:945-966.

12. Chatenoud L: Humoral immune response against OKT3. *Transplant Proc* 1993;25(suppl 1):68-73.

13. Smith TW, Lloyd BL, Spicer N, et al: Immunogenicity and kinetics of distribution and elimination of sheep digoxin-specific IgG and Fab fragments in the rabbit and baboon. *Clin Exp Immunol* 1979;36:384-396.

14. Lotter MG: Measurement of platelet survival and analysis of survival curves, in Heyns A, Badenhorst PN, Lotter MG (eds): *Platelet Kinetics and Imaging,* vol. 1. Boca Raton, FL: CRC Press, 1985.

15. Simon TL, Grace TG: Envenomation coagulopathy in wounds from pit vipers. *N Engl J Med* 1981;305:443-447.

16. Gillissen A, Theakston RDG, Barth J, et al: Neurotoxicity, haemostatic disturbances and haemolytic anaemia after a bite by a Tunisian saw-scaled or carpet viper *(Echis 'pyramidum'*-complex): Failure of antivenom treatment. *Toxicon* 1994;32:937-944.

17. Ho M, Warrell MJ, Warrell D, et al: A critical reappraisal of the use of enzyme-linked immunosorbent assays in the study of snake bite. *Toxicon* 1986;24:211-221.

18. Wenger TL: Experience with digoxin immune Fab (ovine) in patients with renal impairment. *Am J Emerg Med* 1991;9(suppl 1):21-23.

19. Ujhelyi MR, Sylvie R: Pharmacokinetic aspects of digoxin-specific Fab therapy in the management of digitalis toxicity. *Clin Pharmacokinet* 1995;28:483-493.

20. Nakamura RM, Voller A, Bidwell DE: Enzyme immunoassays: Heterogeneous and homogenous systems, in Weir M (ed): *Immunochemistry,* vol 1, ed 4. London: Blackwell Scientific Publications.

Reprint no. **47/1/82579**

Reprints not available from the authors.

APPENDIX: METHODS OF FREE Fab ANTIVENOM AND VENOM ANTIGEN DETECTION

Venom antigen quantitation and free Fab antivenom quantitation were performed by Therapeutic Antibodies, Incorporated. Serum was saved from each blood sample and refrigerated until analysis.

For measurement of venom concentrations, a conventional sandwich ELISA was developed. The capturing antibody (Fab antivenom 5 µg/mL; 100 µg/well in $NaHCO_3$ buffer; .1 mol/L, pH 9.6) was absorbed to microtiter plates overnight at 4° C. After five washes (300 µL/well) with assay buffer (Na_2PO_4 10 mmol/L, NaCl .8%, KCl .02%, Tween-20 .1%, pH 7.4), the plate was incubated with doubling dilutions of the standard (1:1:1:1 mixture of venom from *Crotalus adamanteus, C atrox, C scutulatus,* and *A piscivorus,* 5 mg/L) or sample (100 µg/mL, 100 µL) followed by sheep antihorse antiserum coupled to horseradish peroxidase (1:500, 100 µL) and developed with the use of a substrate solution (o-phenylenediamine; .1% in 0.07 mol/L citrate buffer, pH 5, containing .02% H_2O_2). The reaction was stopped after 10 minutes with sulfuric acid (3 mol/L, 50 µL/well) and the absorbance recorded at 492 nm. Normal sheep serum (.1%) was included in all buffers to reduce nonspecific binding, as proposed by Ho et al.[17] The two antisera used in the ELISA are raised with the use of the four different venoms. The binding to venoms from *C atrox* and *C adamanteus* is higher (range, .005 to 2 mg/L) than that to venom from *C scutulatus* and *A piscivorus* (range, .01 to 10 mg/L).

In another ELISA developed to measure free specific Fab antivenom (ie, that portion of the administered Fab antivenom not bound to venom), microtiter plates were coated with a 1:1:1:1 mixture of venom from *C adamanteus, C atrox, C scutulatus,* and *A piscivorus* (5 µg/mL, 100 µL). The buffers and incubation times described above were used.

After washing, the plate was incubated with doubling dilutions of a Fab antivenom standard (Fab antivenom, 10 mg/L) or samples (100 µL) for 1 hour at 37° C, followed by donkey antisheep antiserum coupled to horseradish peroxidase according to the method of Nakamura et al.[20] Plates were finally developed as described above and the absorbence recorded at 492 nm with the use of a Flow Titertek Multiscan microplate reader. Plasma samples were diluted in assay buffer. The nonspecific binding was low (OD_{492nm}=.08). The minimum detectable Fab antivenom concentration (defined as that producing an effect greater than 2 SDs from 20 determination of zero analyte) was 10 µg/L.

Microtiter plates (Nunc) were obtained from Gibco BRL (Paisley, United Kingdom). Normal sheep serum and donkey antisheep and sheep antihorse sera were purchased from Polyclonal Antibodies, Limited (Dyfed, United Kingdom). Venoms were purchased from Venom Research Laboratory. Crotalid polyvalent antivenom Fab antivenom were obtained directly from the manufacturer, Wyeth. The remaining chemicals were obtained from Sigma Chemical Company (Poole, Dorset, United Kingdom).